PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference BP/G-33319A/SZB	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/EP2004/009321	International filing date *(day/month/year)* 19.08.2004	Priority date *(day/month/year)* 20.08.2003
International Patent Classification (IPC) or national classification and IPC C12P21/02		
Applicant SANDOZ AG et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 7 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ *sent to the applicant and to the International Bureau)* a total of sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
06.05.2005	04.10.2005
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer van de Kamp, M Telephone No. +31 70 340-

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/009321

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-16 as originally filed

Claims, Numbers

1-23 as originally filed

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	7,8,13
	No:	Claims	1-6,9-12,14-23
Inventive step (IS)	Yes:	Claims	7,8,13
	No:	Claims	1-6,9-12,14-23
Industrial applicability (IA)	Yes:	Claims	1-23
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement (Continuation)

1. CITATIONS

Reference is made to the following documents:

D1: HART R A ET AL: "Large scale, in situ isolation of periplasmic IGF-I from E. coli" BIO/TECHNOLOGY, vol. 12, November 1994, pages 1113-1117

D2: EP-A-0 177 343 (GENENTECH INC) 9 April 1986

D3: WO 03/004599 A (PANCER ZEEV ; PELEG YOAV (IL); INSIGHT STRATEGY & MARKETING L (IL)) 16 January 2003

2. NOVELTY (Art. 33(2) PCT)

1. The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of **claims 1-6, 9-12, and 14-23** is not new in the sense of Article 33(2) PCT.

2. **D1** discloses a process for the preparation of recombinant IGF-I produced by *Escherichia coli*, wherein it is secreted into the periplasm, whereby further processing of the fermentation harvest broth is interrupted by a step of solubilisation (cf., e.g., page 1116 right-hand column paragraph 'IGF-I in situ solubilization'), falling within the terms of **claims 1-3, 6, 9, 16-21 and 23**.

3. **D2** discloses a process for the preparation of recombinant human growth hormone by *E. coli*, wherein it is secreted into the periplasm, whereby further processing of the fermentation harvest broth is interrupted by a step of killing the cells (cf., e.g., example 8, and claims 13 and 15), falling within the terms of **claims 1, 6, 9-12 and 16-23**.

4. **D3** discloses a process for the preparation of recombinant human growth

hormone by *E. coli*, wherein it is secreted into the periplasm, whereby further processing of the fermentation harvest broth is interrupted by storage of cells at -20 °C (cf. example 3), falling within the terms of **claims 1-5, 14, 15, and 17-23**.

5. The combination of features of the dependent **claims 7, 8 and 13** with the features of **claim 1** to which they refer is not known from the available prior art. The subject-matter of these claims can therefore be regarded as new in respect of the prior art as defined in the regulations (Rule 64(1)-(3) PCT).

3. INVENTIVE STEP (Art. 33(3) PCT)

1. **D2** is regarded as being the closest prior art to the subject-matter of **claim 1** and discloses a method for recovering a recombinant protein, preferably recombinant human growth hormone, from the periplasmic space of a bacterial cell, preferably *E. coli*, comprising the steps of growing the cells whereby the protein is secreted in the periplasm, killing the cells, and recovering the protein of interest from the cells by a freeze-thaw procedure (cf., example 8, claims 13 and 15). The problem solved by **D2** is the provision of an improved method to recover periplasmic proteins, preferably eukaryotic proteins produced in bacterial hosts, preferably, human growth hormone (cf. page 6 line 33 - page 7 line 12). The step of killing the cells prior to extraction is said to approximately double the product protein recovery without reducing the purity of the product protein in the recovered supernatants (cf. page 21 line 24-26). The disclosure of **D2** renders the subject-matter of **claims 1, 6, 9-12 and 16-23** not novel, and consequently not inventive.

2. Similarly, **D1** and **D3** can be regarded as closest prior art, rendering the subject-matter of **claims 1-3, 6, 9, 16-21 and 23** and of **claims 1-5, 14, 15, and 17-23**, respectively, not novel and consequently not inventive, either.

3. The subject-matter of **claims 7, 8 and 13** in combination with the features of **claim 1** to which they refer, can be regarded as inventive, as they provide solutions to the problem of providing an improved process for the isolation of recombinant proteins expressed in the periplasm of bacterial cells, which are not obvious to the skilled person.

4. INDUSTRIAL APPLICABILITY (Art. 33(4) PCT)

1. The subject-matter of **claims 1-23** satisfies the criterion set forth in Art. 33(4) PCT in conjunction with Rule 5(vi) PCT with respect to industrial applicability.

Re Item VIII
Certain observations on the international application (Continuation)

1. CLARITY (Art. 6 PCT)

1. The use of broad terms in **claim 1** renders the scope of the claim unclear, as it is not clear what may be encompassed by terms such as 'further processing of the fermentation harvest broth' and 'maintaining it under defined conditions'.

2. The subject-matter of **claim 23** is neither clear nor concise, as it seeks to encompass the whole description in a claim. Such claims are not allowable.

2. SUPPORT (Art. 6 PCT)

1. The solution as presented in the current application, particularly referencing to example 1, appears to go against a general prejudice in the field that lengthening of the isolation procedure will result in a *de*crease in the production of recombinant proteins. For this, ample evidence is present in the literature, part of which has been referred to by the applicant in the application. In contrast, based upon the finding that in the case presented

in example 1 the production of a recombinant Fab' with specificity for TNFalpha is *in*creased rather than *de*creased when further processing is interrupted before extraction, a broad **claim 1** has been formulated. It is pointed out that current examples 2 and 3 represent mere assertions that the rhGH and rIFN-alpha 2B extraction yields can be increased by an interruption step.

2. There is sufficient reason to assert that a broad claim such as **claim 1** is not supported over the whole of its scope, and that the invention is not practicable for each and every recombinant protein secreted into the periplasm of a bacterial cell. From the prior art, e.g., as indicated by the applicant in the application, it is apparent to the skilled person that the problem which is dealt with in the current application is not solved for all recombinant proteins by the means offered in the application and referred to in **claim 1**. It is to be expected that the technical effect of increasing the extraction yield of a protein produced in the periplasm of a bacterial cell by including an interruption step prior to extraction, will not be achieved over the whole of the scope of **claim 1**. Henceforth, a lack of support for **claim 1** is noted, contrary to Art. 6 PCT.

3. In line with this reasoning, also the subject-matter of all dependent claims is considered to be unsufficiently supported over the width of the claims.